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# PolySUMO Capture & Release Kit (Cat. # J4410)

This kit is sufficient for purification of polysumoylated proteins from 100 mg or 20 X 5 mg cell or tissue extracts at a concentration of 5 mg/ml.

***Description***

The PolySUMO Capture & Release Kit is designed to rapidly enrich polysumoylated proteins from cell or tissue extracts within 6 hours according to a published method (reference 1) with modifications. This approach uses the SIM domains of RNF4 to capture polysumoylated proteins, which are subsequent released by incubating with a competitive peptide that binds SIM.

***Components***

| Component | Stock | Quantity |
|---|---|---|
| • GST-4XSIM | 4 mg/ml | 1 ml |
| • GST | 4 mg/ml | 1.2 ml |
| • 2X Elution Peptide | 2 X | 0.45 ml |
| • Glutathione Agarose Resin | 50% slurry | 1.5 ml |
| • Iodoacetamine (IAA) | 25 mg | 1 Tube |
| • 20X Cell Lysis Buffer | 20 X | 2 X 1.0 ml |
| • NaCl | 1 M | 4 X 1.25 ml |

***Components in 20X lysis buffer***

- 400 mM Tris, pH 7.6 at 4 $^{0}$C
- 40 mM 2-mecaptoethanol (βME)

***Additional reagents required (not included in the kit)***

- Glycerol, Milli Q water

***To make 50X IAA***

- Reconstitute the supplied IAA powder into 270 µl distilled $H_2O$ or your buffer to make a 100X stock solution at 500 mM.

***To make 20 mL 1X Ccell Lysis Buffer***

| | |
|---|---|
| • 20X Cell Lysis Buffer | 1 ml |
| • 1 M NaCl | 3 ml |
| • 100X IAA | 0.2 ml |
| • Glycerol (not provided) | 2 ml |
| • Milli Q water | 13.6 ml |

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***Protocol***

The following protocol has been used to test the kit for purification polysumoylated proteins from 50 mg HEK 293T whole cell lysates, optimizing the purification conditions may be necessary for your own experiments.

⚠ **All purification steps are operated at 4°C!**

**[Prepare and Lyse cells]**

1. Sixteen dishes (150 mm) of HEK 293 cells were grown in DMEM supplemented with 10% fetal bovine serum to approximately 95% confluence. Cells were harvested and kept in a 50 ml conical tube. Cells can be frozen in -80 °C freezer for future use.
2. Resuspend the cell pellet in 24 ml cell lysis buffer (20 mM Tris, pH 7.6, 150 mM NaCl, 2 mM βME, 5 mM IAA, 10% Glycerol). Briefly sonicate cells using a 550 Sonic Dismembrator (Fisher Scientific). Settings: power output: 2, 20 seconds/time for three times, rest 2 min between sonications.
3. Ultracentrifuge the cell lysates using a 70.1TI rotor (Beckman) at 36,000 rpm for 45min.
4. Carefully transfer the supernatant (avoid the lipids on the top) to a new 50 ml conical tubes. We use a 10 ml syringe with a 21 gauge needle to take the supernatant. Repeat step 3 to further remove lipids if necessary.
5. Measure the cell lysate concentration using the Bradford assay. We obtained a total of 106 mg cell extracts.

**[Bind polysumoylated proteins to GST-4XSIM]**

6. Split the cell extracts in step 5 equally into two parts, 12 ml each in a 15 ml conical tube. In the control reaction, add GST to 0.2 mg/ml; in another tube, add GST-4XSIM to 0.2 mg/ml. Add 200 µL net glutathione agarose resin into each tube (wash 400 µL 50% slurry three times using 2 ml Milli Q water each time to have 200 µL net resin). Mildly rotate the mixtures for 3 hours using a LABQUAKE rotator. More or less GST or GST-4XSIM may be used depending on your cell lysate concentration.

**[Wash Glutathione agarose resin]**

7. After incubation, centrifuge the mixtures using an IEC Centra CL3R centrifuge at 1,000 X*g* for 5 min, discard the supernatant.
8. Add 0.5 ml washing buffer (lysis buffer plus 0.15M NaCl) to the conical tube, transfer resins to a 1.7 ml microcentrifuge tube (try to transfer all resins, multiple rinses and transfers are usually necessary).
9. Rotate the mixtures in step 8 for 3 min using a LABQUAKE rotator, then centrifuge using a desktop centrifuge at 1,000 X*g* for 5 min, discard the supernatant.
10. Use 1.5 ml washing buffer to wash the resins according to step 9 two more times. Discard the supernatant after each centrifugation.

**[Elute polysumoylated proteins]**

11. Resuspend the washed glutathione resins into 100 µl cell lysis buffer, add 100 µl 2X elution peptide. Mildly rotate or rock the mixtures for 60 min.



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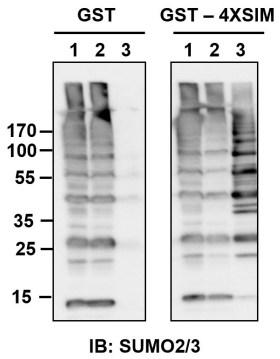
12. Centrifuge the mixtures in step 11 using a desktop centrifuge at 1,000 X*g* for 5 min. Carefully transfer the supernatant into a new 0.65 ml microcentrifuge tube. Rinse the glutathione agarose resins with 200 µl lysis buffer and repeat the centrifugation step, combine the supernatant with the first elution.

**[Quantification]**

13. 20 µl cell lysates before and after GST or GST-4XSIM binding and 5 µl eluate were separated on a SDS-PAGE and immunoblotted with an antibody recognizing SUMO2 and SUMO3 (see attached Figure).




Lane 1: Cell lysates before pulldown

Lane 2: Cell lysates after pulldown

Lane 3: Elution

**[References]**

Bruderer R, et al. Purification and identification of endogenous polySUMO conjugates. (2011) EMBO Rep. 12(2):142-8.